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(54) Title: FROZEN FOOD PRODUCT CONTAINING ANTI-FREEZE PEPTIDES

(57) Abstract

A frozen food product comprising AFP, wherein the fractional ice phase volume in the product is below 0.27.

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FROZEN FOOD PRODUCT CONTAINING ANTI-FREEZE PEPTIDES

Technical Field of the Invention

The invention relates to food products containing Anti-freeze peptides (AFPs), in particular to frozen food products containing AFPs.

Background to the Invention

Anti-freeze peptides (AFPs) have been suggested for improving the freezing tolerance of foodstuffs. In particular it has been suggested that some AFPs may be capable of increasing the smooth texture of aerated frozen food products such as ice cream. Up till now, however the use of AFPs has not been applied to commercially available food products. One reason for this is that up till now it has proved difficult to reproducibly produce a frozen food product having the desired texture and eating characteristics.

Applicants believe that one of the possible reasons for the lack of desired texture in frozen food products containing AFP is that although the AFP is capable of recrystallisation inhibition it is often not capable of avoiding the formation of brittle textures. Applicants believe that one of the explanations for this is that AFPs seem capable of controlling the particle size of the ice-crystals. However the presence of AFP may also lead to an adverse effect in that the ice-crystals tend to form aggregates leading to hard and brittle products.

Surprisingly it has now been found that if the properties of the frozen food material are carefully chosen, this leads to an improved texture e.g. a reduced hardness and/or brittleness of the product. In particular applicants have found that if the ice phase volume of the frozen food product is carefuly chosen this leads to a product of reduced brittleness.

Accordingly in a first aspect the present invention relates to a frozen food product comprising AFP, wherein the fractional ice phase volume in the product is below 0.27.

Background to the invention

For the purpose of this inventions the term AFP has the meaning such as well-known in the art, see for example "Antifreeze proteins and their potential use in frozen food products", Marilyn Griffith and K. Vanya Ewart, Biotechnology Advances, Vol 13, pp 375-402, 1995.

Anti-freeze peptides have been described in various literature places. Also these literature places suggest their use in food products, but normally no actual indications are given how to prepare food products of good quality on an industrial scale.

The above mentioned article by Griffith et.al. suggests to use AFPs in foods that are eaten while frozen by inhibiting recrystallisation and maintaining a smooth texture. However no clear guidelines are given how to obtain this smooth texture. Applicants have found that despite the fact that AFPs can indeed inhibit recrystallisation, the use of AFPs

in food products often lead to brittle textures and not the smooth textures as indicated in the Griffith article.

WO 90/13571 discloses antifreeze peptides produced chemically or by recombinant DNA techniques from plants. The AFPs can suitably be used in food-products such as ice cream. Again no guidelines are given as to how to obtain smooth textures.

WO 92/22581 discloses AFPs from plants, which can be used for controlling ice crystal growth in ice cream. This document also describes a process for extracting a polypeptide composition from intercellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

The present invention aims at providing the food manufacturer a greater flexibility of using AFP material in frozen food products when aiming to obtain a product with improved recrystallisation properties in combination with a good texture. In particular it has been found that the texture of frozen food products containing AFPs can markedly be improved by carefully controlling the phase volume of the ice in the product.

Frozen food products contain a certain amount of ice, usually present in the form of ice-crystals. The fractional phase volume of the ice is that part of the volume of the frozen product that is filled with ice. The ice phase volume can be calculated, such as for example illustrated in the examples.

Applicants have now found that surprisingly a relatively low phase volume of the ice in the frozen product containing AFP, favourably contributes to the textural properties of said product. Also applicants have surprisingly found that low ice phace volumes in frozen products with AFP may lead to improved firmness, creamy texture and crumbliness as compared to the same products with a high ice phase volume.

Preferably the fractional ice phase volume in products of the invention is less than 0.27, more preferred less than 0.26, most preferred less than 0.25. Generally the ice phase volume will be more than 0.05, more generally more than 0.10, most preferred more than 0.15. The fractional ice-phase volume is preferably determined at the storage temperature of the frozen food.

The appropriate levels for the fractional ice phase volume in the frozen product can be achieved by several suitable methods e.g. by adapting the processing route, the formulation and/or the storage conditions of the frozen food product. It is believed to be well within the ability of the skilled person to select those conditions which lead to the desired phase volume of the ice.

Examples of suitable measures to be taken to influence the ice phase volume are:

By increasing the storage temperature of the frozen food product, this generally leads to a reduction in the ice phase volume of the product. Therefore in one preferred embodiment of the invention the AFP containing food product

is stored at relatively high temperatures say from -2 °C to -15 °C, more preferred from -6 to -14 °C, or -10 to -12 °C.

By increasing the phase volume of non-frozen elements in the composition, the ice phase volume can also be reduced. For example compositions of the invention may be aerated to increase the phase volume of air an correspondingly reduce the phase volume of the ice-crystals.

Also product re-formulation may be used to reduce the ice phase volume in the product. For example the amount of fat may be increased or the amount of other ingredients which tend to be present in the non-frozen phase (e.g. proteins or fillers such as maltodextin). Also ingredients may be added which lead to lowering of the freezing point of the composition such as alcohols like glycol or sugars or salts leading to a reduction of the ice phase volume.

It will be well within the ability of the skilled person to select from the above and other possible measures those which alone or in combination provide the desired ice phase volume.

Frozen food products of the invention may be any food product which can be stored and/or eaten in the frozen state. Examples of frozen food products which may contain AFPare processed food products such as for example frozen bakery products e.g. doughs, batters, cakes etc, frozen culinary products for example soups, sauces, pizzas, frozen vegetable products such a compote, mashed potato, tomato paste etc. A very preferred food product according to the invention is a frozen confectionery product.

For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees. Especially preferred products of the invention are ice-cream and water-ice.

Frozen products according to the invention are preferably aerated. Preferably the level of aeration is more than 50%, more preferably more than 70%, most preferable more than 90%. Generally the level of aeration will be less than 400%, more general less than 300, most preferred less than 200%. As described above an increase in the level of aeration may be used to decrease the ice phase volume in the frozen product.

Preferably the level of AFPs in the frozen food product of the invention is from 0.0001 to 0.5 wt% based on the final product.

The AFP for use in products of the invention can be any AFP suitable for use in food products. Examples of suitable sources of AFP are for example given in the above mentioned article of Griffith and Vanya Ewart.

The AFP's can be obtained from their sources by any suitable process, for example the isolation processes as described in the above mentioned documents.

One possible source of AFP materials is fish. Examples of fish AFP materials are AFGP (for example obtainable from

Atlantic cod, Greenland cod and Tomcod), Type I AFP (for example obtainable from Winter flounder, Yellowtail flounder, Shorthorn sculpin and Grubby sculpin), Type II AFP (for example obtainable from Sea raven, Smelt and Atlantic herring) and Type III AFP (for example obtainable from Ocean out, Atlantic wolffish, Radiated shanny, Rock gunnel and Laval's eelpout). A preferred example of the latter type is described in WP 97/02343.

Another possible source of AFP material are invertebrates. Also AFPs may be obtained from Bacteria.

A third possible source of AFP material are plants. Examples of plants containing AFPs are garlic-mustard, blue wood aster, spring oat, winter cress, winter canola, Brussels sprout, carrot, Dutchman's breeches, spurge, daylily, winter barley, Virginia waterleaf, narrow-leaved plantain, plantain, speargrass, Kentucky bluegrass, Eastern cottonwood, white oak, winter rye, bittersweet nightshade, potato, chickweed, dandelion, spring and winter wheat, triticale, periwinkle, violet and grass.

Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in accordance to the present invention.

Genetic manipulation techniques may be used to produce AFPs. Genetic manipulation techniques may be used to produce AFPs having at least 80%, more preferred more than 95%, most preferred 100% homology to the AFP's directly

obtained from the natural sources. For the purpose of the invention these AFPs possessing this high level of homology are also embraced within the term "AFPs".

The genetic manipulation techniques may be used as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (eg in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

For the purpose of the invention preferred AFPs are derived from fish or plants. Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as

described in our case WO 97/02343. From plants especially the use of AFPs from carrot (see PCT/EP97/06181) or grass (see PCT/EP97/03634) are preferred.

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

Preferably those AFPs are chosen which have significant ice-recrystallisation inhibition properties. This can be measured in accordance to example I.

Preferably AFPs in accordance to the invention provide an ice particle size upon recrystallisation -as measured in accordance to the examples- of less than 20 µm, more preferred from 5 to 15 µm.

Preferably the level of solids in the frozen food product (e.g. sugar, fat, flavouring etc) is more than 2 wt%, more preferred from 4 to 70wt%.

The method of preparing the frozen food product of the invention can be selected from any suitable method for the preparation of frozen food products. The AFP's can generally be added at various stages of the preparation, for example it can be added in the first pre-mix of ingredients or can later be added during a later stage of the preparation process. For some applications it is sometimes preferred to add the AFP's at a relatively late stage of the production process, for example after (partial) pre-freezing of the product.

The freezing process of the invention will generally include the freezing of the composition say to a temperature of less than -2 °C, say from -80 to -5 °C. If desired, products of the invention do not need to be subjected to low temperatures to avoid ice-crystal growth. Therefore the products may for example be frozen without the need to use low temperatures say less than -25°C and can also be stored at temperatures which are higher than traditional temperatures to store frozen confectionery products. For some applications a storage temperature of -2 to -15 °C, for example -10 to -14 °C may be advantageous, for other applications, storage at lower temperatures e.g. -15 °C or less, particularly from -16 to -60 °C, e.g. around -18 °C may be preferred.

For some applications it may be advantageous to include a mixture of two or more different AFPs into the food product. One reason for this can for example be that the plant source for the AFP's to be used, contains more than one AFP and it is more convenient to add these, for example because they are both present in the AFP source to be used. Alternatively it may sometimes be desirable to add more than one AFP from different sources.

The invention will now be illustrated by means of the following examples.

Example I

Method of determining whether an AFP possesses ice recrystallisation inhibition properties.

Recrystallisation inhibition properties can measured using a modified "splat assay" (Knight et al, 1988). 2.5 µl of the solution under investigation in 30% (w/w) sucrose is transferred onto a clean, appropriately labelled, 16 mm circular coverslip. A second coverslip is placed on top of the drop of solution and the sandwich pressed together between finger and thumb. The sandwich is dropped into a bath of hexane held at -80°C in a box of dry ice. When all sandwiches have been prepared, sandwiches are transferred from the -80°C hexane bath to the viewing chamber containing hexane held at -6°C using forceps pre-cooled in the dry ice. Upon transfer to -6°C, sandwiches can be seen to change from a transparent to an opaque appearance. Images are recorded by video camera and grabbed into an image analysis system (LUCIA, Nikon) using a 20x objective. Images of each splat are recorded at time = 0 and again after 30-60 minutes. The size of the ice-crystals in both assays is compared. If the size at 30-60 minutes is similar only moderately (less than 10%) increased compared to the size at t=0, this is an indication of good ice-crystal recrystallisation properties.

Also if the ice-crystal size number (average length) is less than 20 µm, preferably from 5-15 µm this is a sign of good ice-crystal recrystallisation inhibition properties. The ice-crystal size can conveniently be determined by

highlighting the crystals manually and drawing around the perimeter. Images of the highlighted crystals can then be measured using image analysis software which counts the number of pixels to complete the longest straight line (length), shortest straight line (breadth) and the aspect ratio (length/breadth). The number average length is used as particle size.

Example II

Pre-mixes for preparing ice-cream were made by mixing:

Formulation wt%	1	2	3	4	5	6
Butteroil	9.5	9.5	9.5	9.5	9.5	9.5
Milk Protein	18.5	18.5	18.5	18.5	18.5	18.5
Sucrose	12.2	12.2	10.2	10.2	7.4	7.4
Glucose Syrup	5.0	5.0	14.5	14.5	29.2	29.2
Mono glycerol palmitate	0.33	0.33	0.33	0.33	0.33	0.33
Guar gum	0.11	0.11	0.11	0.11	0.11	0.11
Locust Bean gum	0.11	0.11	0.11	0.11	0.11	0.11
Vanillin	0.01	0.01	0.01	0.01	0.01	0.01
AFP*	-	0.005	-	0.005	-	0.005
Water	to balance					
calculated fractional ice phase volume at -18°C	0.30	0.30	0.27	0.27	0.22	0.22

* Note AFP (Type III HPLC-12) as described in WO 97/02343

The mixes can be used in the preparation of a ice-cream by homogenisation at 2000 psi and 65 °C followed by ageing over night at 5oC. The mix was frozen using a freezer (MF50 SSHE Technohoy fitted with a solid dasher rotating 240 rpm) The extrusion temperature was -4.5°C, the overrun was 100%. The product is then frozen at -35°C and stored at -18°C.

Ice phase volumes were calculated by first calculating the amount of water that will freeze. This is done by the following method:

(a) For each ingredient, a plot is contructed showing freezing point depression against concentration of the ingredient in water;

(b) The amount of water bound by the ingredient at temperature T can then be calculated as:

$$Wi = (100/Ci - 1) * Si$$

whereby Ci is the concentration of the ingredient i that is required to depress the freezing temperature of water to the temperature T and Si is the concentration of the ingredient in the formulation.

(c) the calculation under (b) is repeated for each ingredient in the formulation and the total amount of water bound can then be calculated by adding up all the values Wi.

(d) the amount of water that will freeze can then be calculated by subtracting the total amount of water bound from the total amount of water in the formulation.

The rest of the calculation can then be done as follows (the formulas below are somewhat simplified, but sufficiently accurate to calculate the fractional ice phase volume of products of the invention):

1. Using the above method the amount of water (wt%) that will freeze at -18 °C can be calculated.

2. The volume fraction of the ice in the unaerated product can then be calculated as:

(p) (wt% of water that will freeze * density of total unaerated mix) / (density of ice * 100)

3. Similarly the initial volume fraction of the water that becomes ice can be calculated as:

(q) (wt% of water that will freeze * density of total mix)/ 100.

4. The total volume factor (wrt the unfrozen product) of the frozen unaerated product can then be calculated as:

(r) volume fraction of ice + (1 - volume of water that becomes ice) = (p) + (1 - (q))

5. The fractional ice phase volume of the unaerated product can be calculated by dividing the result of (p) by the result of (r):

(s) (p) / (r)

6. The fractional air phase volume can be calculated by:

(t) overrun %/ (100 + overrun%)

7. The fractional ice phase volume in the aerated product can then be calculated by:

(u) (1 - fractional air phase volume) * fractional ice phase volume of unaerated product = (1 - (t)) * (s)

Hardness measurements were made on the 6 formulations described above by using a Hounsfield HTE hardness tester at -18°C using a 10 mm diameter probe penetrating a block of the ice-cream at a rate of 400 mm/min to a depth of 20 mm. In these measurements an increased hardness corresponds to increased brittleness.

The following results were obtained:

Ice content @ -18°C (%)	Type III AFP level (%wt)	Hardness @ -18°C (N)
52	0	11.8 ± 3.0
	0.005	47.1 ± 12.1
45	0	7.2 ± 1.2
	0.005	25.3 ± 3.5
35	0	4.5 ± 1.1
	0.005	9.3 ± 2.6

This example clearly illustrates that if AFPs are added this leads to a clear increase of the brittleness of the product. By adding high levels of maltodextrin to the composition, a lower ice phase volume can be obtained. The examples show that if the fractional ice phase volume is below 0.27, surprisingly an acceptable brittleness can be obtained even in the presence of AFP.The above products 2, 4 and 6 were also subjected to sensory analysis. Products 2 and 4 scored significantly lower than product 6 on firmness, creamy texture and crumbliness. Again it was therefore confirmed that a lower ice phase volume leads to improved properties.

Example III

This example illustrates the method of calculation of the amount of water that will freeze for the following model formulation at -20°C :

Lactose	5.83
Sucrose	15.0
Water	79.17

(I) The amount of water prevented from freezing by sucrose

$$W_{sucrose} = ((100 / C_{sucrose}) - 1) * S_{sucrose}$$

From the plot of freezing point depression against solute concentration it can be seen that at -20°C, $C_{sucrose} = 70\%$

Therefore the amount of water prevented from freezing by sucrose is:

$$W_{sucrose} = ((100 / 70) - 1) * 15.0 = 6.43$$

(II) Similarly the amount of water prevented from freezing by lactose

$$W_{lactose} = ((100 / C_{lactose}) - 1) * S_{lactose}$$

From the plot of freezing point depresiion against solute concentration it can be seen that at -20°C, $C_{lactose} = 69\%$

Therefore the amount of water prevented from freezing =

$$W_{lactose} = ((100 / 69) - 1) * 5.83 = 2.62$$

The the total amount of water to be frozen (ice) is equal to:

$$W - W_{sucrose} - W_{lactose} = 79.17 - (6.43 + 2.62) = 70.12 \text{ wt\%}$$

Claims

1. A frozen food product comprising AFP, wherein the fractional ice phase volume in the product is below 0.27.

2. A frozen food product according to claim 1, wherein the fractional ice phase volume is from 0.05 to 0.26.

3. A frozen food product according to claim 1, wherein the ice phase volume is measured at -18 °C.

4. A frozen food product according to claim 1, wherein the ice phase colume is measured at a temperature between -2 and -15 °C.

5. A frozen food product according to claim 1, being a frozen confectionery product.

6. A frozen food product according to claim 1, wherein the level of AFP is from 0.0001 to 0.5 wt%.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23G9/02 A23L3/37

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23G A23C A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	WO 97 02343 A (UNILEVER) 23 January 1997 see claims 1,5-7 ---	1,2,4-6
A	EP 0 427 544 A (FUJI OIL) 15 May 1991 see column 1, line 1 - line 9; examples ---	1
A	WO 97 02754 A (UNILEVER) 30 January 1997 see page 1, line 22 - page 2, line 30; claim 1; example 1 see page 3, line 5 - line 6 ---	1
A,P	WO 97 30599 A (UNILEVER) 28 August 1997 --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 02567 A (AULT FOODS) 18 February 1993 see page 23, line 35 - page 24, line 4; claim 1 see page 26, line 37 - page 27, line 20 see page 1, line 15 - line 20 see page 2, line 6 - page 3, line 9	1
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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